CLAIMS

We claim:

1. A crystal comprising a catalytic domain of Syk protein or homologue thereof.

2. The crystal according to claim 1, further comprising a chemical entity, wherein said chemical entity binds to the catalytic domain of Syk protein or homologue thereof.

3. The crystal according to claim 2, wherein said chemical entity binds to an active site on the catalytic domain of Syk protein or homologue thereof.

4. The crystal according to claim 3, wherein the chemical entity is selected from the group consisting of staurosporine, adenosine, ATP, an ATP analogue, a nucleotide triphosphate, a nucleotide diphosphate, phosphate and active site inhibitor.

5. The crystal according to claim 3, wherein the chemical entity is selected from the group consisting of staurosporine and AMP-PNP.

6. The crystal according to claim 2, wherein the chemical entity binds to a substrate binding site on the catalytic domain of Syk protein or homologue thereof.

7. The crystal according to claim 6, wherein the chemical entity is selected from the group consisting of NAc-Glu-Glu-Asp-Asp-Tyr-Glu-Ser-Pro-NH_2 (SEQ ID NO: 2), Glu-Glu-Asp-Asp-Tyr-Glu-Ser-Pro (SEQ ID NO: 5), a peptide comprising the amino acid sequence Glu-Asp-Asp-Tyr (residues 2-5 of SEQ ID NO:5), a peptide comprising the

amino acid sequence Asp-Asp-Tyr-Glu (residues 3-6 of SEQ ID NO:5), a peptide comprising the amino acid sequence Asp-Tyr-Glu-Ser (residues 4-7 of SEQ ID NO:5), a peptide comprising the amino acid sequence Tyr-Glu-Ser-Pro (residues 5-8 of SEQ ID NO:5), a peptide comprising the amino acid sequence Glu-Glu-Asp-Asp-Tyr (residues 1-5 of SEQ ID NO:5), a peptide comprising the amino acid sequence Glu-Asp-Asp-Tyr-Glu (residues 2-6 of SEQ ID NO:5), a peptide comprising the amino acid sequence Asp-Asp-Tyr-Glu-Ser (residues 3-7 of SEQ ID NO:5), a peptide comprising the amino acid sequence Asp-Tyr-Glu-Ser-Pro (residues 4-8 of SEQ ID NO:5), a peptide comprising amino acids Asp-Glu-Glu-Asp-Tyr (SEQ ID NO:6), a peptide comprising amino acids Asp-Glu-Glu-Tyr-Asp (SEQ ID NO:7), a peptide comprising amino acids Asp-Glu-Tyr-Glu-Asp (SEQ ID NO:8), a peptide comprising amino acids Asp-Tyr-Glu-Glu-Val (SEQ ID NO:9), and a peptide comprising amino acids Tyr-Ser-Ile-Ile-Nle (SEQ ID NO:10).

8. The crystal of claim 1 or 2, wherein said catalytic domain of Syk protein or homologue thereof is phosphorylated.

9. The crystal according to claim 1 or 2, wherein said catalytic domain of Syk protein is selected from the group consisting of amino acid residues 343-635, amino acid residues 358-635 and amino acid residues 364-634 of SEQ ID NO: 1.

10. The crystal according to claim 1 or 2, wherein said catalytic domain of Syk protein comprises amino acid residues 343-635 of SEQ ID NO: 1.

11. A crystallizable composition comprising a catalytic domain of Syk protein or homologue thereof.

12. The crystallizable composition according to claim 11, further comprising a chemical entity, wherein said chemical entity binds to the catalytic domain of Syk protein or homologue thereof.

13. The crystallizable composition according to claim 12, wherein said chemical entity binds to an active site on the catalytic domain of Syk protein or homologue thereof.

14. The crystallizable composition according to claim 13, wherein the chemical entity is selected from the group consisting of staurosporine, adenosine, ATP, an ATP analogue, a nucleotide triphosphate, a nucleotide diphosphate, phosphate and active site inhibitor.

15. The crystallizable composition according to claim 13, wherein the chemical entity is selected from the group consisting of staurosporine and AMP-PNP.

16. The crystallizable composition according to claim 12, wherein the chemical entity binds to a substrate binding site on the catalytic domain of Syk protein or homologue thereof.

17. The crystallizable composition according to claim 16, wherein the chemical entity is selected from the group consisting of NAc-Glu-Glu-Asp-Asp-Tyr-Glu-Ser-Pro-NH_2 (SEQ ID NO: 2), Glu-Glu-Asp-Asp-Tyr-Glu-Ser-Pro (SEQ ID NO: 5), a peptide comprising the amino acid sequence Glu-Asp-Asp-Tyr (amino acids 2-5 of SEQ ID NO:5)

a peptide comprising the amino acid sequence Asp-Asp-Tyr-Glu (residues 3-6 of SEQ ID NO:5), a peptide comprising the amino acid sequence Asp-Tyr-Glu-Ser (residues 4-7 of SEQ ID NO:5), a peptide comprising the amino acid sequence Tyr-Glu-Ser-Pro (residues 5-8 of SEQ ID NO:5), a peptide comprising the amino acid sequence Glu-Glu-Asp-Asp-Tyr (residues 1-5 of SEQ ID NO:5), a peptide comprising the amino acid sequence Glu-Asp-Asp-Tyr-Glu (residues 2-6 of SEQ ID NO:5), a peptide comprising the amino acid sequence Asp-Asp-Tyr-Glu-Ser (residues 3-7 of SEQ ID NO:5), a peptide comprising the amino acid sequence Asp-Tyr-Glu-Ser-Pro (residues 4-8 of SEQ ID NO:5), a peptide comprising amino acids Asp-Glu-Glu-Asp-Tyr (SEQ ID NO:6), a peptide comprising amino acids Asp-Glu-Glu-Tyr-Asp (SEQ ID NO:7), a peptide comprising amino acids Asp-Glu-Tyr-Glu-Asp (SEQ ID NO:8), a peptide comprising amino acids Asp-Tyr-Glu-Glu-Val (SEQ ID NO:9), and a peptide comprising amino acids Tyr-Ser-Ile-Ile-Nle (SEQ ID NO:10).

18. The crystallizable composition of claim 11 or 12, wherein said catalytic domain of Syk protein or homologue thereof is phosphorylated.

19. The crystallizable composition according to claim 11 or 12, wherein said catalytic domain of Syk protein is selected from the group consisting of amino acid residues 343-635, amino acid residues 358-635 and amino acid residues 364-634 of SEQ ID NO: 1.

20. The crystallizable composition according to claim 11 or 12, wherein said catalytic domain of Syk protein comprises amino acid residues 343-635 of SEQ ID NO: 1.

21. A computer comprising:

(a) a machine-readable data storage medium, comprising a data storage material encoded with machine-readable data, wherein said data defines the binding pocket or domain selected from the group consisting of:

(i) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues Asp494, Gly532, Lys533, Trp534 and Pro535 according to Figure 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(ii) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues L377, M424, V433, M448, A451, G454, L501 and S511 according to Figure 1 or 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(iii) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues L377, G378, S379, V385, A400, K402, V433, M448, E449, M450, A451, E452, P455, R498, N499, L501, S511 and D512 according to Figure 1 or 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(iv) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues K375, E376, G380, N381, G383, T384, K386, K387, T398, V399, V401, M424, R434, L446, V447, L456, K458, D494, A496, A497, V500, L502, V503, K509, I510, F513 and G514 according to Figure 1, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(v) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues D376, G380, G383, T384, K386, T398, V399, V401, L417, E420, M424, R434, M435, L446, V447, L453, G454, L456, N457, D494, A497, V500, L502, V503, K509, I510, F513, G514 and L515 according to Figure 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å; and

(vi) a set of amino acid residues which are identical to Syk amino acid residues according to Figure 1 or 2, wherein the root mean square deviation of the backbone atoms between said amino acid residues and said Syk amino acid residues which are identical is not greater than about 5 Å;

(b) a working memory for storing instructions for processing said machine-readable data;

(c) a central processing unit coupled to said working memory and to said machine-readable data storage medium for processing said machine-readable data and a

means for generating three-dimensional structural information of said binding pocket or domain; and

(d) output hardware coupled to said central processing unit for outputting three-dimensional structural information of said binding pocket or domain, or information produced using said three-dimensional structural information of said binding pocket or domain.

22. The computer according to claim 21, wherein the binding pocket is produced by homology modeling of the structure coordinates of said Syk amino acid residues according to Figure 1 or 2.

23. The computer according to claim 21, wherein said means for generating three-dimensional structural information is provided by means for generating a three-dimensional structural representation of said binding pocket or domain.

24. The computer according to claim 21, wherein said output hardware is a display terminal, a printer, CD or DVD recorder, ZIP™ or JAZ™ drive, a disk drive, or other machine-readable data storage device.

25. A method of using a computer for selecting an orientation of a chemical entity that interacts favorably with a binding pocket or domain selected from the group consisting of:

(i) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues Asp494, Gly532, Lys533, Trp534 and Pro535 according to Figure 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said

Syk amino acid residues which are identical is not greater than about 3 Å;

(ii) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues L377, M424, V433, M448, A451, G454, L501 and S511 according to Figure 1 or 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(iii) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues L377, G378, S379, V385, A400, K402, V433, M448, E449, M450, A451, E452, P455, R498, N499, L501, S511 and D512 according to Figure 1 or 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(iv) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues K375, E376, G380, N381, G383, T384, K386, K387, T398, V399, V401, M424, R434, L446, V447, L456, K458, D494, A496, A497, V500, L502, V503, K509, I510, F513 and G514 according to Figure 1, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(v) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk

amino acid residues D376, G380, G383, T384, K386, T398, V399, V401, L417, E420, M424, R434, M435, L446, V447, L453, G454, L456, N457, D494, A497, V500, L502, V503, K509, I510, F513, G514 and L515 according to Figure 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å; and

(vi) a set of amino acid residues which are identical to Syk amino acid residues according to Figure 1 or 2, wherein the root mean square deviation of the backbone atoms between said amino acid residues and said Syk amino acid residues which are identical is not greater than about 5 Å;

said method comprising the steps of:

(a) providing the structure coordinates of said binding pocket or domain on a computer comprising the means for generating three-dimensional structural information from said structure coordinates;

(b) employing computational means to dock a first chemical entity in the binding pocket or domain;

(c) quantitating the interaction energy between said chemical entity and all or part of the binding pocket or domain for different orientations of the chemical entity; and

(d) selecting the orientation of the chemical entity with the most favorable interaction energy.

26. The method according to claim 25, further comprising generating a three-dimensional graphical representation of the binding pocket or domain prior to step (b).

27. The method of claim 25, wherein energy minimization with or without molecular dynamics simulations or rigid-body minimizations are performed simultaneously with or following step (b).

28. The method according to claim 25, further comprising the steps of:

(e) repeating steps (b) through (d) with a second chemical entity; and

(f) selecting at least one of said first or second chemical entity that interacts more favorably with said binding pocket or domain based on said quantitated interaction energy of said first or second chemical entity.

29. A method of using a computer for selecting an orientation of a chemical entity with a favorable shape complementarity in a binding pocket selected from the group consisting of:

(i) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues Asp494, Gly532, Lys533, Trp534 and Pro535 according to Figure 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(ii) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues L377, M424, V433, M448, A451, G454, L501 and S511 according to Figure 1 or 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(iii) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues L377, G378, S379, V385, A400, K402, V433, M448, E449, M450, A451, E452, P455, R498, N499, L501, S511 and D512 according to Figure 1 or 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(iv) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues K375, E376, G380, N381, G383, T384, K386, K387, T398, V399, V401, M424, R434, L446, V447, L456, K458, D494, A496, A497, V500, L502, V503, K509, I510, F513 and G514 according to Figure 1, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(v) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues D376, G380, G383, T384, K386, T398, V399, V401, L417, E420, M424, R434, M435, L446, V447,

L453, G454, L456, N457, D494, A497, V500, L502, V503, K509, I510, F513, G514 and L515 according to Figure 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å; and

(vi) a set of amino acid residues which are identical to Syk amino acid residues according to Figure 1 or 2, wherein the root mean square deviation of the backbone atoms between said amino acid residues and said Syk amino acid residues which are identical is not greater than about 5 Å;

said method comprising the steps of:

(a) providing the structure coordinates of said binding pocket and ligand bound therein on a computer comprising the means for generating three-dimensional structural information from said structure coordinates;

(b) employing computational means to dock a first chemical entity in the binding pocket;

(c) quantitating the contact score of said chemical entity in different orientions; and

(d) selecting an orientation with the highest contact score.

30. The method according to claim 29, further comprising generating a three-dimensional graphical representation of the binding pocket and ligand bound therein prior to step (b).

31. The method according to claim 29, further comprising the steps of:

(e) repeating steps (b) through (d) with a second chemical entity; and

(f) selecting at least one of said first or second chemical entity that has a higher contact score based on said quantitated contact score of said first or second chemical entity.

32. A method for identifying a candidate inhibitor of a molecule or molecular complex comprising a binding pocket or domain selected from the group consisting of:

(i) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues Asp494, Gly532, Lys533, Trp534 and Pro535 according to Figure 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(ii) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues L377, M424, V433, M448, A451, G454, L501 and S511 according to Figure 1 or 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(iii) a set of amino acid residues comprising at least four amino acid residues which are

identical to Syk amino acid residues L377, G378, S379, V385, A400, K402, V433, M448, E449, M450, A451, E452, P455, R498, N499, L501, S511 and D512 according to Figure 1 or 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(iv) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues K375, E376, G380, N381, G383, T384, K386, K387, T398, V399, V401, M424, R434, L446, V447, L456, K458, D494, A496, A497, V500, L502, V503, K509, I510, F513 and G514 according to Figure 1, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(v) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues D376, G380, G383, T384, K386, T398, V399, V401, L417, E420, M424, R434, M435, L446, V447, L453, G454, L456, N457, D494, A497, V500, L502, V503, K509, I510, F513, G514 and L515 according to Figure 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å; and

(vi) a set of amino acid residues which are identical to Syk amino acid residues according to Figure 1 or 2, wherein the root mean square deviation of the backbone atoms between said amino acid residues and said

Syk amino acid residues which are identical is not greater than about 5 Å;

comprising the steps of:

(a) using a three-dimensional structure of the binding pocket or domain to design, select or optimize a plurality of chemical entities;

(b) contacting each chemical entity with the molecule or the molecular complex;

(c) monitoring the inhibition to the catalytic activity of the molecule or molecular complex by each chemical entity; and

(d) selecting a chemical entity based on the inhibitory effect of the chemical entity on the catalytic activity of the molecule or molecular complex.

33. A method of designing a compound or complex that interacts with a binding pocket or domain selected from the group consisting of:

(i) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues Asp494, Gly532, Lys533, Trp534 and Pro535 according to Figure 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(ii) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues L377, M424, V433, M448, A451, G454, L501 and S511 according to Figure 1 or 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and

said Syk amino acid residues which are identical is not greater than about 3 Å;

(iii) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues L377, G378, S379, V385, A400, K402, V433, M448, E449, M450, A451, E452, P455, R498, N499, L501, S511 and D512 according to Figure 1 or 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(iv) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues K375, E376, G380, N381, G383, T384, K386, K387, T398, V399, V401, M424, R434, L446, V447, L456, K458, D494, A496, A497, V500, L502, V503, K509, I510, F513 and G514 according to Figure 1, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å;

(v) a set of amino acid residues comprising at least four amino acid residues which are identical to Syk amino acid residues D376, G380, G383, T384, K386, T398, V399, V401, L417, E420, M424, R434, M435, L446, V447, L453, G454, L456, N457, D494, A497, V500, L502, V503, K509, I510, F513, G514 and L515 according to Figure 2, wherein the root mean square deviation of the backbone atoms between said at least four amino acid residues and said Syk amino acid residues which are identical is not greater than about 3 Å; and

(vi) a set of amino acid residues which are identical to Syk amino acid residues according to Figure 1 or 2, wherein the root mean square deviation of the backbone atoms between said amino acid residues and said Syk amino acid residues which are identical is not greater than about 5 Å;

comprising the steps of:

(a) providing the structure coordinates of said binding pocket or domain on a computer comprising the means for generating three-dimensional structural information from said structure coordinates;

(b) using the computer to dock a first chemical entity in part of the binding pocket or domain;

(c) docking at least a second chemical entity in another part of the binding pocket or domain;

(d) quantifying the interaction energy between the first or second chemical entity and part of the binding pocket or domain;

(e) repeating steps (b) to (d) with another first and second chemical entity, selecting a first and a second chemical entity based on said quantified interaction energy of of all of said first and second chemical entity;

(f) optionally, visually inspecting the relationship of the first and second chemical entity to each other in relation to the binding pocket or domain on a computer screen using the three-dimensional graphical representation of the binding pocket or domain and said first and second chemical entity; and

(g) assembling the first and second chemical entity into a compound or complex that interacts with said binding pocket or domain by model building.

34. A method of utilizing molecular replacement to obtain a structural model of a molecule or a molecular complex of unknown structure, comprising the steps of:

(a) crystallizing said molecule or molecular complex;

(b) generating an X-ray diffraction pattern from said crystallized molecule or molecular complex;

(c) applying at least a portion of the structure coordinates set forth in Figures 1, 2 or a homology model thereof to the X-ray diffraction pattern to generate a three-dimensional electron density map of at least a portion of the molecule or molecular complex whose structure is unknown; and

(d) generating a structural model of the molecule or molecular complex from the three-dimensional electron density map.

35. The method according to claim 34, wherein the molecule is selected from the group consisting of a Syk catalytic domain protein and a Syk catalytic domain homologue.

36. The method according to claim 34, wherein the molecular complex is selected from the group consisting of a Syk catalytic domain protein complex and a Syk catalytic domain homologue complex.